

IN THE CLAIMS

1. (original) A modified nucleotide or nucleoside molecule comprising a purine or pyrimidine base and a ribose or deoxyribose sugar moiety having a removable 3'-OH blocking group covalently attached thereto, such that the 3' carbon atom has attached a group of the structure



wherein Z is any of $-C(R^{IV})_2-O-R''$, $-C(R')_2-N(R'')$, $-C(R')_2-N(H)R''$, $-C(R^{IV})_2-S-R''$ and $-C(R')_2-F$, wherein $-C(R^{IV})_2-O-R''$ is of the formula $-CR^4(R^5)-O-CR^4(R^5)-OR^6$ or of the formula $-CR^4(R^5)-O-CR^4(R^5)-SR^6$; and wherein $-C(R^{IV})_2-S-R''$ is of the formula $-CR^4(R^5)-S-CR^4(R^5)-OR^6$ or of the formula $-CR^4(R^5)-S-CR^4(R^5)-SR^6$;

wherein each R'' is or is part of a removable protecting group;

each R' is independently a hydrogen atom, an alkyl, substituted alkyl, arylalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclic, acyl, cyano, alkoxy, aryloxy, heteroaryloxy or amido group, or a detectable label attached through a linking group; or $(R')_2$ represents an alkylidene group of formula $=C(R''')_2$ wherein each R''' may be the same or different and is selected from the group comprising hydrogen and halogen atoms and alkyl groups;

each R⁴ and R⁵ is independently a hydrogen atom or an alkyl group;

R⁶ is alkyl, cycloalkyl, alkenyl, cycloalkenyl or benzyl; and

wherein said molecule may be reacted to yield an intermediate in which each R'' is exchanged for H or, where Z is $-C(R')_2-F$, the F is exchanged for OH, SH or NH₂, preferably OH, which intermediate dissociates under aqueous conditions to afford a molecule with a free 3'OH; with the proviso that where Z is $-C(R^{IV})_2-S-R''$, both R^{IV} groups are not H.

2. (original) A molecule according to claim 1 wherein R' is an alkyl or substituted alkyl.

3. (currently amended) A molecule according to claim 1 ~~or claim 2~~ wherein -Z is of formula $-C(R')_2-N_3$.

4. (currently amended) A molecule according to ~~any one of claims~~ claim 1 to 3 wherein Z is an azidomethyl group.
5. (currently amended) A molecule according to claim 1 ~~or claim 2~~ wherein R'' is a benzyl or substituted benzyl group.
6. (currently amended) A molecule according to ~~any preceding~~ claim 1 wherein said base is linked to a detectable label via a cleavable linker or a non-cleavable linker.
7. (original) A molecule according to claim 6 wherein said linker is cleavable.
8. (currently amended) A molecule according to ~~any one of claims~~ claim 1 to 5 wherein a detectable label is linked to the molecule through the blocking group by a cleavable or non-cleavable linker.
9. (currently amended) A molecule according to ~~any one of claims~~ claim 6 to 8 wherein said detectable label is a fluorophore.
10. (currently amended) A molecule according to ~~any one of claims~~ claim 6 to 9 wherein said linker is acid labile, photolabile or contains a disulfide linkage.
11. (currently amended) A modified nucleotide molecule as claimed in ~~any one of claims~~ claim 1 to 10 which comprises one or more ³²P atoms in its phosphate portion.
12. (original) A nucleoside, nucleotide or polynucleotide molecule of formula PN-O-allyl, wherein PN is said nucleoside or nucleotide or is a 3'terminal nucleotide of said polynucleotide;

and said nucleoside or nucleotide further comprises in addition to the allyl blocking group a detectable label linked to the base thereof by a cleavable or non-cleavable linker.

13. (original) A molecule according to claim 12 wherein said linker is cleavable.

14. (currently amended) A molecule according to claim 12 ~~or claim 13~~ wherein said detectable label is a fluorophore.

15. (currently amended) A molecule according to ~~any one of claims~~ claim 12 ~~to 14~~ wherein said linker is acid labile, photolabile or contains a disulfide linkage.

16. (original) A method of converting a compound of formula R-O-allyl, R₂N(allyl), RNH(allyl), RN(allyl)₂ or R-S-allyl to a corresponding compound in which the allyl group is removed and replaced by hydrogen, said method comprising the steps of reacting a compound of formula R-O-allyl, R₂N(allyl), RNH(allyl), RN(allyl)₂ or R-S-allyl in aqueous solution with a transition metal comprising a transition metal and one or more ligands selected from the group comprising water-soluble phosphine and water-soluble nitrogen-containing phosphine ligands, wherein the or each R is a water-soluble biological molecule.

17. (original) The method of claim 16 wherein said compound is of formula R-O-allyl.

18. (currently amended) The method of claim 16 ~~or claim 17~~ wherein said R is part of a nucleoside, a nucleotide or a polynucleotide molecule.

19. (original) The method of claim 18 wherein said nucleoside, nucleotide or polynucleotide further comprises a detectable label linked to the base thereof by a cleavable or non-cleavable linker.

20. (original) A molecule according to claim 19 wherein said linker is cleavable.
21. (currently amended) The method of claim 19 ~~or claim 20~~, wherein said detectable label is a fluorophore.
22. (currently amended) The method of ~~any one of claims~~ claim 19 ~~to 21~~ wherein said linker is acid labile, photolabile or contains a disulfide linkage.
23. (currently amended) The method of ~~any one of claims~~ claim 19 ~~to 22~~ wherein said allyl group and said label are removed in a single step.
24. (currently amended) The method of ~~any one of claims~~ claim 16 ~~to 23~~ wherein said transition metal is selected from the group comprising platinum, palladium, rhodium, ruthenium, osmium and iridium.
25. (currently amended) The method of ~~any one of claims~~ claim 16 ~~to 24~~ wherein said transition metal is palladium.
26. (currently amended) The method of ~~any one of claims~~ claim 16 ~~to 25~~ wherein said group of ligands comprise derivatised triaryl phosphine ligands or derivatised trialkyl phosphine ligands.
27. (currently amended) The method of ~~any one of claims~~ claim 16 ~~to 26~~ wherein said group of ligands are derivatised with one or more functionalities selected from the group comprising amino, hydroxyl, carboxyl and sulfonate groups.
28. (currently amended) The method of ~~any one of claims~~ claim 16 ~~to 27~~ wherein the group

of ligands comprises 3,3',3''-phosphinidynetris (benzenesulfonic acid) and tris(2-carboxyethyl)phosphines and their salts.

29. (currently amended) A method of controlling the incorporation of a nucleotide as defined in ~~any one of claims~~ claim 6 to 10 or as defined in ~~any one of claims 12 to 15~~ and complementary to a second nucleotide in a target single-stranded polynucleotide in a synthesis or sequencing reaction comprising incorporating into the growing complementary polynucleotide said nucleotide, the incorporation of said nucleotide preventing or blocking introduction of subsequent nucleoside or nucleotide molecules into said growing complementary polynucleotide.

30. (original) The method of claim 29, wherein the incorporation of said nucleotide is accomplished by a terminal transferase or polymerase or a reverse transcriptase.

31. (original) The method of claim 30 wherein the polymerase is a *Thermococcus* sp.

32. (original) The method of claim 31 wherein the *Thermococcus* sp is 9^oN or a single mutant or double mutant thereof.

33. (original) The method of claim 32 wherein the double mutant is -Y409V A485L.

34. (currently amended) A method for determining the sequence of a target single-stranded polynucleotide, comprising monitoring the sequential incorporation of complementary nucleotides, wherein at least one incorporation is of a nucleotide as defined in ~~any one of claims~~ claim 6 to 10 or as defined in ~~any one of claims 12 to 15~~ and wherein the identity of the nucleotide incorporated is determined by detecting the label linked to the base, and the blocking group and said label are removed prior to introduction of the next complementary nucleotide.

35. (original) The method of claim 34 wherein the label of the nucleotide and the blocking group are removed in a single chemical treatment step.

36. (currently amended) A method for determining the sequence of a target single-stranded polynucleotide, comprising:

(a) providing a plurality of different nucleotides wherein said plurality of different nucleotides are ~~either as defined in any one of claims~~ claim 6 to 10 ~~or as defined in any one of claims 12 to 15~~ and wherein the detectable label linked to each type of nucleotide can be distinguished upon detection from the detectable label used for other types of nucleotides;

(b) incorporating the nucleotide into the complement of the target single-stranded polynucleotide;

(c) detecting the label of the nucleotide of (b), thereby determining the type of nucleotide incorporated;

(d) removing the label of the nucleotide of (b) and the blocking group; and

(e) optionally repeating steps (b)-(d) one or more times;

thereby determining the sequence of a target single-stranded polynucleotide.

37. (currently amended) The method of claim 36 wherein said incorporating ~~incorporating~~ step is accomplished by a *Thermococcus sp.*

38. (original) The method of claim 37 wherein the *Thermococcus sp* is 9^oN or a single mutant or double mutant thereof.

39. (original) The method of claim 38 wherein the double mutant is -Y409V A485L.

40. (currently amended) The method of ~~any one of claims~~ claim 36 to 39 wherein the label of the nucleotide and the blocking group are removed in a single chemical treatment step.

41. (currently amended) A method according to ~~any one of claims~~ claim 36 to 40, wherein each of the nucleotides are brought into contact with the target sequentially, with removal of non-incorporated nucleotides prior to addition of the next nucleotide, and wherein detection and removal of the label and the blocking group is carried out either after addition of each nucleotide, or after addition of all four nucleotides.

42. (currently amended) The method according to ~~any one of claims~~ claim 36 to 40, wherein each of the nucleotides are brought into contact with the target together simultaneously, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and the blocking group.

43. (currently amended) The method according to ~~any one of claims~~ claim 36 to 40, comprising a first step and a second step, wherein in the first step, a first composition comprising two of the four nucleotides is brought into contact with the target and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and wherein in the second step, a second composition comprising the two nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group, and wherein the first and second steps are optionally repeated one or more times.

44. (currently amended) The method according to ~~any one of claims~~ claim 36 to 40, comprising a first step and a second step, wherein in the first step, a composition comprising one of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein in the second step, a second composition comprising the three nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides

are removed prior to detection and subsequent to removal of the label and blocking group and wherein the first steps and the second step are optionally repeated one or more times.

45. (currently amended) The method according to ~~any one of claims~~ claim 36 to 40, comprising a first step and a second step, wherein in the first step, a first composition comprising three of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein in the second step, a composition comprising the nucleotide not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein the first steps and the second step are optionally repeated one or more times.

46. (currently amended) A kit, comprising:

(a) a [a] plurality of different nucleotides wherein said plurality of different nucleotides are ~~either as defined in any one of claims~~ claim 6 to 10 ~~or as defined in any one of claims 12 to 15; and~~

(b) packaging materials therefor.

47. (original) A kit according to claim 46, wherein the detectable label in each nucleotide can be distinguished upon detection from the detectable label used for any of the other three types of nucleotide.

48. (currently amended) The kit of claim ~~46 or 47~~, further comprising an enzyme and buffers appropriate for the action of the enzyme.

49. (canceled)

50. (original) A method of using a nucleotide of claim 1 wherein said method includes a Sanger or Sanger-type sequencing method.
51. (new) A method of controlling the incorporation of a nucleotide as defined in claim 12 and complementary to a second nucleotide in a target single-stranded polynucleotide in a synthesis or sequencing reaction comprising incorporating into the growing complementary polynucleotide said nucleotide, the incorporation of said nucleotide preventing or blocking introduction of subsequent nucleoside or nucleotide molecules into said growing complementary polynucleotide.
52. (new) A method for determining the sequence of a target single-stranded polynucleotide, comprising monitoring the sequential incorporation of complementary nucleotides, wherein at least one incorporation is of a nucleotide as defined in claim 12 and wherein the identity of the nucleotide incorporated is determined by detecting the label linked to the base, and the blocking group and said label are removed prior to introduction of the next complementary nucleotide.
53. (new) A method for determining the sequence of a target single-stranded polynucleotide, comprising:
- (a) providing a plurality of different nucleotides wherein said plurality of different nucleotides are as defined in claim 12 and wherein the detectable label linked to each type of nucleotide can be distinguished upon detection from the detectable label used for other types of nucleotides;
 - (b) incorporating the nucleotide into the complement of the target single-stranded polynucleotide;
 - (c) detecting the label of the nucleotide of (b), thereby determining the type of nucleotide incorporated;
 - (d) removing the label of the nucleotide of (b) and the blocking group; and
 - (e) optionally repeating steps (b)-(d) one or more times;
- thereby determining the sequence of a target single-stranded polynucleotide.

54. (new) A kit, comprising:
- (a) a plurality of different nucleotides wherein said plurality of different nucleotides are as defined in claim 12; and
 - (b) packaging materials therefor.
55. (canceled)